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TITLE: Chemotherapy Agents and the Inhibition of Neuronal Birthing in the Brain - The Cause of "Chemo Brain"

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**13. ABSTRACT (Maximum 200 Words)**

Patients undergoing chemotherapy can experience a decline in cognitive abilities. While well described from a clinical perspective, little is known of the neurological substrate for this difficulty, commonly known as 'chemo brain.'

We hypothesize that the cognitive difficulties experienced by patients undergoing chemotherapy are the result of impaired neurogenesis, especially in the hippocampus. We further hypothesize that agents that do not cross the blood-brain barrier will not show reduced rates of neurogenesis, in contrast to agents that readily cross into the central nervous system (CNS). Our objective is to compare the effect of drugs that enter the CNS (Cytoxan and 5-FU) with agents that do not (Adriamycin and Taxol) with respect to their ability to impair the birthing of new neurons in the hippocampus of adult mice.

By testing whether chemotherapeutic agents that enter the CNS can reduce neurogenesis, we hope to develop an animal model of 'chemo brain' that will allow further studies. Furthermore, if we can show that inhibition of neurogenesis is a correlate of behavioral decline after chemotherapy, we will have provided evidence that modification of chemotherapeutic regimens – specifically, using strategies to prevent CNS entry of drugs – would be of great importance in improving the quality of life in cancer patients.

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**INTRODUCTION:**

The purpose of the work was to test the hypothesis that chemotherapeutic agents can cause a reduction in neuronal birthing in the hippocampus. The rationale for this proposition is that the hippocampus is a crucial structure for memory function; as such, any disruption to its normal functioning – including disruption of the normal process of neuronal birthing – could lessen memory function. Such a process could underlie the condition popularly known as "chemo brain," in which persons who have received chemotherapy have reduced cognitive skills. To test this hypothesis, we chose chemotherapeutic agents of two groups, those that cross the blood-brain barrier () and those that do not (); we predict, based on our hypothesis, that those that readily enter the brain would reduce neuronal birthing, whereas there would be no effect on birthing by those agents that do not enter the brain. Mice were to be treated with these agents, and bromo-deoxy-uridine (BrdU) would be injected, thereby labeling newly birthed cells. By use of immunohistochemical techniques, we sought to determine the number of birthed neurons and their destiny – for example, differentiation into neurons or glia.

**REPORT:**

*Extenuating Circumstances:* The progress has been slow due to unforeseen circumstances. The original technician hired was not able to leave his previous employment until a few months into the funding period; as he had all the skills necessary to do the work, we waited, as it would have taken at least that long to hire and train another individual. Unfortunately, he left unexpectedly (to enter an MD/PhD program), and it proved difficult to find another person with the requisite skill set. In addition, we were using the imaging facilities of a collaborator, who unexpectedly left this institution, leaving us temporarily without adequate microscopy support. The original pilot experiment, designed to test the safety and efficacy of the chosen chemotherapeutic regimens was finally completed, but with results that could not be interpreted with certainty (below). A second pilot was delayed until the proper technical support was in place. The second pilot is just now being finished. We have asked for and received a twelve-month extension to continue this work.

*Report:* To date, the original pilot has been completed. Groups of mice (4 per treatment group, 12 saline control) were treated with either diluent or one of the following chemotherapeutic agents on days 1, 4 and 7: Taxol (5 mg/kg); 5-FU (100 and 60 mg/kg), the lower dose being chosen due to toxicity; Cytoxan (50 mg/kg); or adriamycin (5mg/kg). In the group receiving the higher 5-FU dose, ¾ died. In the remaining treatment groups, none died or lost weight. Mice were then injected with four doses (50 mg/kg i.p.) of BrdU, two hours apart, one day after the completion of the chemotherapeutic regimen. We chose this BrdU paradigm as our initial work in this area suggested that one day dosing of BrdU might be sufficient to measure cell birthing. Animals were sacrificed at day 36, 28 days after the BrdU injections. Tissue was fixed *in vivo* and processed for chromagen staining with biotin-avidin. Fluorescent labeling was planned in a later stage using tissue that was sectioned but reserved. Chromagen staining was performed on every third hippocampal slice.

While this experiment suggested that the chemotherapeutic regimens chosen were relatively safe – in other words, that toxicity would not be a major confound – we failed to determine whether the chemotherapeutic drugs altered neuronal birthing. This is because we underestimated the amount of BrdU needed. With this labeling procedure, we counted 0-4 labeled cells per hippocampal slice; we sampled every third slice of the ~50 per hippocampus (at 50 μm), but even the totals were insufficient to determine a chemotherapy drug's effect. Indeed, there was overlap among controls and treated groups: controls, 10 BrdU-positive cells/18 slices (range, 0-33); Cytoxan, 27 (2-37); Taxol, 48 (43-57); adriamycin, 10 (8-12); and 5-FU, 7 (0-12).

Part of the variability may be biological, but in reviewing the notebooks, we noted that the IHC procedure varied from our usual protocol (the technician who did this work has left our institution and was not readily available to address this)(see references). Additionally, we may have overestimated the counts expected with BrdU injections over a single day. Related to this, our protocol called for sacrifice of the animals 28 days after the BrdU injections. The rationale for this was to allow time for differentiation to neurons or glia. However, this reduces cell counts due to cell death.

To address the first concern, we have begun using positive controls, tissue obtained from a collaborator who measures neuronal birthing in neonatal animals (much higher birthing rates). This will serve as a check on our IHC protocols (corrected for the deviation from protocol).

To address the concern that we have insufficiently labeled our adult tissue, we have embarked on a second pilot, with the same chemotherapeutic regimens but with *four* days of labeling (4 injections per day on days 8-11) with BrdU; animals were sacrificed at day 21, 15 days sooner than in the initial experiment and only 10 days after the BrdU injections. To conserve resources and to use time efficiently, we had only control, Taxol and 5-FU groups in this series. At the time of writing, the tissue is being sliced and stained. If can assure ourselves that our protocol is reproducible (using the neonatal tissue), then we will process the tissue from this experiment for cell counting. If the expected numbers of BrdU-positive cells are seen in controls, then we will proceed to process the experimental animals and complete the injections in the remaining chemotherapeutic groups.

**REPORTABLE OUTCOMES:**

None to date

**CONCLUSIONS:**

Our results to date are insufficient to address our hypothesis, that chemotherapeutic agents may cause "chemo brain" because of an effect on neuronal birthing in the hippocampus. We have identified technical concerns and are addressing these in a systematic manner (above).

The hypothesis remains testable and important: if we can show an effect on neuronal birthing, a number of potential discoveries follow. For example, mice appropriately treated to have reduced neuronal birthing could be tested behaviorally, to gain an understanding of the deficits associated

with changes in neuronal birthing rates. Strategies that reduce penetration of chemotherapeutic agents into the CNS may allow adequate systemic treatment, but without the psychological-behavioral consequences.

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